Studies On Growth Inhibitory And Antibiofilm Activities Of Actinomycetes Against Noscomoial *Candida Albicans* Strains

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STUDIES on Growth Inhibitory &AntibiofilmAntibiofilm Activities of ActinomycetesAgainst Nosocomial *Candida albicans* Strains

Abstract :-

In recent era, the demand for novel anti fungal therapies has increased many folds due to its potential to treat several biofilm associated intfections. Candida bioflm is a complex association of hyphal cells that are associated with both biotic & abiotic surrfaces.C.albicans biofilm infection is an invasive fungal infection &acts as an important virulent factor. The challenges linked with biofilm associated infections prompted researchers to discover factors responsible for inhibition of growth & biofilm formation. The resistance of fungal biofilms to antifungal agents is complex &multifactortal which makes most available antifungals ineffective against C.albicans biofilms because high concentrations are required or significant side effects are observed due to toxicity.The secondary metabolites of actinomycetes are rich in active substances capable of inhibiting growth&biofilm formation in many pathogenic microorganisms.Actinomycetes from various soil &marine ecosystems are considered as unique resources of secondary metabolites in last few decades. The terrestrial envirnoments are unique in their soil physicochemical parameters cultivated plant varieties&microflora. However in last few decades the frequency of isolating redundant se condary metabolites from similar strains has been documented several times.In addition to this during process of isolation, many actinbacteria may be escaped due to their slow growth rate orinadequte availability of essesntial growth nutrients. Keeping all these aspects in view present study is planned to isolate active metabolite producing actionomycetes from diverse environments & using various selective methods which can target biofilm formation by nocosomial strains of C.albicans.Biobilm assays light&scanning electron microscopy studies will be carried out to detect morphological changes in *C. albicans*& advanced methods will be used to find antibiofilmmechanism.The active metabolites will be characterized by FIIR,NMR & GC-MS analysis.The study will higlight the theoretical significance for development of antifungal drugs&anti candida bilofilm agents.

Introduction

The microbial community in a biofilm is structurally organized group of microorganisms thatare irreversibly attached to surfaces of implanted devices, inert material, or living tissue and areproducer of extracellular polymers that provide a protective structural matrix (Lewis kim (2001) Dolan RM (2001) Themicroorganisms in biofilms are characterized with lower growth rates and higher resistance toantimicrobial treatment, thus behaving very differently from planktonic cells (Dolan RM 2001) The ability toadhere to different types of surfaces enables microorganisms to form biofilm on medical devices,including intravascular catheters, prosthetic heart valves and joint replacements, or in differenttissues in the host (Douglas LJ 2003) linking biofilms to persistent colonization and infections Poter 1999 About 65 % of all human microbial infections are linked with biofilm formation. In recent era,the prevalence of fungal infections has been increased accounting for over 10 lakhs of humandeaths annually. The increasing onset of fungal infections has become delinquent health issuethroughout the globe. Based on severity and complications, these mycotic infections are rangedfrom non life threatening mucocutaneous illnesses to intrusive infections that can affect any organ(Pfaller and Diekema, 2007; Yapar, 2014). Among the mycotic infections, *Candida albicans* caused infections contribute for more than 40% of mortality rates. It is a stereotypical fungalpathogen in humans, and asymptomatically associated with normal flora of host. However, itbecomes invasive and virulent when converted to a hyphal form covered with extracellularpolymeric substance.According to National Nosocomial infection Survey (NNIS), Candida spp ranked fourth most

common cause of nosocomial bloodstream infections during 1990s. In recent studies, it has beenfound that Candida spp are third most frequent nosocomial bloodstream isolates (Perlroth et al.,2007; Lohse et al., 2018). Although various Candida species have emerged as importantpathogens, C.albicansremains the most isolated species, equipped with a broad range ofvirulence factors and commensal characteristics that confer an ability to colonize and invade hosttissue. These factors include the expression of adhesins and invasins on the cell surface, ability todamage host cells, contact sensing, phenotypic switching, secretion of hydrolytic enzymes, andformation of biofilms [Calderone RA, 2001;9:327–35. Mayer FL,. Virulence. 2013;4(2):119–28, Höfs S, Mogavero S, Hube B. Interaction of Candida albicans with host cells: virulence factors, host defense, escape strategies, and the microbiota. JMicrobiol. 2016;54(3):149–69].

The high resistance of Candida biofilms to conventional treatments is associated to severalissues: (i) drug sequestration by the matrix; (ii) up-regulation of drug efflux pumps; (iii) presenceof multidrug-tolerant persister cells (Taff, H. T. et el (2013).Conventional antifungal therapies typically contrast the symptoms of planktonic cells releasedfrom the biofilm, but fail to kill the biofilm itself; thus, after cycles of antifungal therapy, biofilminfections are typically recurring until surgically removed. Hence, novel antifungals must bedeveloped with low toxicity and high therapeutic activity. The secondary metabolites ofactinomycetes are rich in active substances. Recently, actinomycetes strains have also been shownto inhibit *C. albicans* bioflm formation. For example, Guo et al. found that three types of oralactinomycetes supernatants infuenced cell adhesion, cell proliferation, mycelium growth, andmetabolic enzyme activity (protease and phospholipase) and inhibited the formation of *C. albicans* bioflms [Guo YQ, etel (2015) Arch Oral Biol 60:1368–1374.]. In 2016, Srivastava et al. found that the crude extract of ethylacetate from Streptomyces coronicum ADP4 inhibited the formation of bioflms by reducing cell adhesion and transforming the mycelium morphology [. Srivastava V, Dubey AK (2016) Antibiofilmactivity of the metabolites of *Streptomyces chrestomyceticus* strain ADP4 against *Candida albicans.* J BiosciBioeng 122:434–440.]. Cordova-Davalos et al. reported that planktonic *C.albicans* cells were destroyed with the application of a 98% ethanol extract of Streptomyces gcal25, which changed the transformation from yeast to mycelia and induced abnormal cellmorphology (cell contraction) to inhibit biofilm formation [Cordova-Davalos LE, EscobcdoChavez KG, EvangelistaMartinez Z (2018) Inhibition of Candida albicans cell growth and biofilmformation by a bioactive extract produced by soil Streptomyces strain GCAL-25. Arch BiolSci70:387–396.]. The above literature indicates that isolation of unique actinomycetes isolates thatcan produce abundant secondary metabolites from various environments can lead for developmentof novel bioactive metabolites.

In this study, inhibition of growth and biofilm formation by *C. albicans* was used as a target toscreen more active actinomycetes strains isolated from the various soil environments. Biofilmassays, optical microscopy, and scanning electron microscopy (SEM) were used to evaluate biofilm inhibition and detect morphological changes in *C. albicans.* The active secondary metabolites will be characterized by advanced analytical methods as per the need. This study provides a theoretical basis for the research and development of antifungal drugs and anti-*C.albicans* bioflm agents.

Review of literature:-

Lacret et al conducted the study using rhizospheric soil, The antifungal activity of streptoomyces strains may be due to their ability to produce some bioactive secondary metabolites such as isoikarugamycin, novel polycyclic tetramic acid macrolactam produced *by Streptomyces zhaozhuyensis* active againt *C. albicans.*

Hera Nirwati,etel (2022) According to another study actnomyctes isolated from soil Streptomyces spp. GMR22 has remarkable potential as antifungal and antibiofilm againt *C.albicans*. and the active compound was palmitic acid. (1)

Diana Escalantz-Rendiz, Susana de la Roaa Garcia, Raul Tapia Tussel, Jesus Martin, Fernando Reyes, Francisca Vicentle, &MarxelaGaboa-Angulo. Studies conducted using organic extract of streptomyasparvisporgones isolated from Mexican tropical soil indicated the presence of known antifungal compound carbazomycinbl as the major component.

Mohamad Sharifi&NirichanKunchirman- The study conducted using man grove soil as a source of actinomycetes *Streptomyces viridocromogenes* by 16s RNA analysis. S.viridocromogenes has been reported for production of oligopeptide antibiotic compound. The present isolate MB showed significant activity against different pathogenic Candida species.

In another study preformed using coal mine soil the actiomyctes strain is olated BHPL-KSK05. It was given in a study that there strain was found to devlop a flexous spore chain with a smooth surface have higher antifungal activity.

T Janaki BK Nayak& T Ganesan performed screening mangrove actinomycetes for anti Candida activity. They isolated. *Streptomyces cacoi* & Observed that it produce pyrimidine & polypeptide nucleoside based antibiotics for controlling *C. albicans.*

Objective of the study :-

- Isolation of actinomycetes from terrestrieal ecosystem.
- Isolation and antibiotic susceptibility testing of *C. albicans.*

- Evaluation of biofilm formation potential of *C. albicans.*

- Screening and evaluation of bioactive metabolite producing actinomycetes for antimicrobial and antibiofilm activities against *C. albicans.*

  - Identification of isolate at molecular level.
  - Production and optimization of bioactive compound.
  - Characterization of bioactive compound.
  - Quantification of antibiofilm activity of bioactive compound.

Methodology to be adopted:-

1) **Isolation of** *C.albicans*:-

- Isolation of *C.albicans* will be carried out from various nosocomial sources including hospital environments &infected patients samples..Reference strain will be procured from MTCC.

2) **Isolation of Actinomycetes:**

- Isolation of actinomycetes from different terrestrial ecosystems in &around Nanded, Marathwadaregion(Maharashtra, India) will be carried out using selective medias & soil treatment procedures.

3) **Biofilm assay of C.albicans:**

- The screening of isolated *C.albicans* to form biofilm to be carried out using standard microtitre plate assay.(OTOOL)

4) **Screening of antibiotic resistance in samples:-**

- Evaluation of antibiotic resistance among isolated *C.albicans* strains will be carried out using dics diffusion broth dilution &agar well diffusion methods against commercially available antifungal antibiotics.

- Screening of actinomycetes isolates for their growth &biofilm inhibition potential against selected will be carried out using agar well diffusion assay & broth dilution assay.

5)Morphological and molrcularcharacterisation :-

- The potential actinomycetes isolates will be identified on the basis of morphological &biochemical, molecular characteristics.

6) Production of bioactive compounds :-

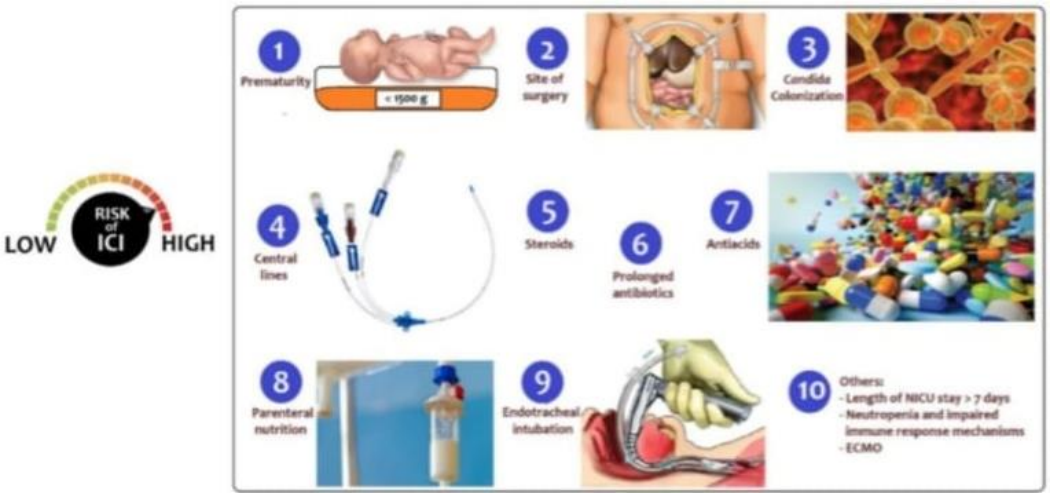
- Production of bioactivemetabolitesfrom selected actinomycetes strains in will be carried out in optimized mredia(based on various cultural & nutritional parametres)by submerged fermentation methods.

7)Extraction&purification:-

- Extraction &purification of produced antifungal metabolite will be carried out using solvent extraction &column chromatography procedures**.**
- **8)Characterisation of purified metabolite:-**
- Characterisation of purified metabolite will be carried out using analytical procedures such as FTIR,NMR,GC-MS analysis.
- 9)**Quantification of antifungal activity:-**
- Quantification of antifungal activity&antibiofilm activity will be done by MFC &MBIC as well as effects will be studied by SEM analysis.

IMPORTANCE:-C. albicans is an opportunistic pathogen causing systemic infections in immuno compromised patients. *C albicans* can stay longer is host & can be aided by drug resistance traits wiich often cause failure& therapeutic strategies. Biofilm formation is one of the importance virulence factor in candida pathogenesis & hospital acquired infections are often related to the ability to produce biofilm on mucosal surfaces &implanted medical devices.By considering this efforts being made to develop alternative strategies to combat growth & biofilm inhibition in *C albicans;* the present study focuses on is isolating bioactive actinimycetes strains capable of inhibiting candida biofilms.Actinomyces have proven track record of producing a wide array a bioactive metabolites. The proposed research work is planned to study the

inhibitory effects of bioactive extracts from actinomycetes on growth&biofilm formation in *C.albicans*.The study may be helpful in development of novel antifungal&antibiofilm drugs against Candida infections.



1) International Status :-

- In other countries meta analysis carried out aimed to analyze Candida biofilm rate, type & antifungal resistant among hospitilized patients between 1995 & 2020. In this study they concluded that early detection of biofilms & a better characterization of Candida spp. blood stream infections should be considered, which eventually will help preserve public health resources & ultimately diminish mortality among patients. (Maria Belen Carrera, fausto Sebastian CabezasMera, EduroTejera Antonio Machado - Prevalence of bilfilms in Candida spp bloodstream infections : A meta analysis)

- In another study, They studied the in vitro Antimicrobial properties of Coconut oil on Candida specias (Ibada Nigeria). They isolated *C.albicans* from clinical specimes. They determined the effectiveness of virgin coconut oil as an antifungal agent on these species. They concluded that coconut oil was active against species of Candida at 100% concn compared to other antibiotics. They recommended use of coconut oil in the treatment of fungal infectins in view of emerging drug resistant Candida species. (DacidolusogaOgoboet el, 2007)

- In one study, It was given that genus Candida can cause both superficial & serious systemic disease & they recognized as major agents of hospital acquired infection. They found

that many Candida infections involve the formation of biofilms on implanted devices such as indwelling catheters or prosthetic heart valves. They studies suggested that with mixed biofilms containing Candida & bacterial species suggested that externsive& striking interactions occour between the prokaryotic & eukaryotic cell in these adherent populations. (L. Julia Douglas Candida biofilms & their role in infection)

- In another study, in which biofilm formation by *C.albicans* was studied. It Indicated that Hwp1 is required for normal biofilm formation. (Clarissa J. Nobile, Jeniel E. Nelt, David David R. Andes, Aron P. Mitcheli Function of *Candida albicans* Adhesin Hwp1 in Biofilm formation)

2) National Status :-

- In India it was revealed the alarming increase in infections with multidrug resistant bacteria is due to overuse of a broad spectrum antimicrobials which leads to over growth of Candida Spp. Thus enhancing its opportunity to use the disease. (P.Bhattacharjee, 2016)

- One study which aimed to identify &speciate Candida isolates from bloodstream infection & evaluate their epidemological profile & antibiotic susceptibility pattern in a tertiary care hospital in North India. They conclude that *C.albicans* predominated in bloodstream infections & were found to be more resistant to antifungals.

According to the data provided by Center for Diseases control & Prevention (CDC) & the National Healthcare Safety Network (NHSN), Candida Species ranked 10 th among hospital acquired pathogens & 5th among nosocomial BSI Pathogens after *S.aurus*, Cogulase negative *Staphylococcus,Klebsiellaspp & Enterococus faecalis.* (GarimaGautam, etel 2019)

- In recent Study which was carried out to retrospectively analyze the distribution of Candida in various samples, their special types & susceptibility in a tertiary care hospital, in India for 4 years. They gave results as the occurrence level had increased successively over the years & they concluded that such study might guide clinicians to select an apporopriate antifungal therapy & plan effective strategies to control invasive & systemic Candida infections. (Uma Maheshwari, etel 2023)

Proposed work plan:-

First year of the project:-

- Completion of the course work.
- Topic selection & finalization
- References to be studied.
- Isolation of Actinomyces spp. Maintaine of culture for future use.
- Isolation of *C.albicans.* Maintaine of culture for further use .

Second Year of Project:-

Screening of isolated Actinomycetes spp.

- Molecular characterisation of Actinomycetecsspp.

Screening of *C.albicans* isolates for biofilm formation potential

Third Year of The Project :

- Testing antifungal,antibiofilm activity of the crude extract using standard methods& comparison with standard antifungal medicine at different concentration.
- Testing drug resistance,biofilm formation potential of *C.albicans*.
- Genome assay& Molecular characterisation of streptomyces will be carried out.
- Result & conclusion of all the procedures to be compared with standard references.
- Preparation& submission of research articles to the reputed national also international journals to be done.
- Compilation& analysis of data.
- Submissionof thesis to the university.

REFERENCES :-

1) Hera Nirwati,EmaDamayanti,EtiNurweningSholikhahMustaofaMutofa,JakaWidada(Heliyon vol.8,4April2022 eo9333)
2) VartikaSrivastva& Ashok K.Dubey(Div of biotechnology NSIT New Delhi.India) (2016)
3) Diana Escalantz-Rendiz,Susana de la Roaa Garcia,Raul Tapia Tussel,Jesus Martin,Fernando Reyes,Francisca Vicentle,&MarxelaGaboa-Angulo (2019)
4) Tracy Miller,Diana Elizabeth Watutangi&Yogiara (2022)
5) S K Augustine,SP Bhavsar,MBasaeriasalehi& BP Kappadnis(Dept.0f Microbiology University of Pu ne India) (2004)

6) Mohamad Sharifi & Nirichan Kunchirman (2019)
7) Mary Sulakshana Palla,Giriija Shankar Guntuku,Prafulla Kumar Sahu, Padmaja Kota,Jagdish Panda (2020)
8) William R Jarvis, clinical infections diseases 20 (6), 1526 – 1530, 1995
9) Joshua perloth, Bryan Choi, Brad spellberg medical my cology 45(P), 321-346, (2007)
10) George J. Alangaden MD Infections Eepidemiology, Infection control, & Prevention.(2010)
11) TanuAtriwal, KashishAzeem, fohadmabood Hussain, Afzal Hussain, Muhammed Nadeem Khan, Mohamed F. Asami Mohamad Abid (mechanistic understanding of C.albicans Biofilm formation approaches for its inhibition) (2021)
12) Sheevani, Poonam Sharma &ArunaAggrawal(Nosocomial candid infection in a rural tertiary care Hospital) (2013)
13) Lacretet. al. The study conducted using rhizophere soil
14) Sheng – giving yang, Hui-jiezhou, Hong zeng (25Sept 2022)
15) T Janaki, BK Nayak& T Ganesan (The pharmainnovation)
16) Vijaykumar, BinduNaik, OmprakashGusain, GajrajS.Bist. (An actinomyce isolate from solitary wasp mud nest) (2014)

18) P. Bhattacharjee (Epidemiology & antifungal susceptibility of canida specious in atertiary care hospital, Kolkata, India) (2016)
19) GarimaGautam, DeeptiRawat, Ravinder Kaur &MasomNathaniCandidemia Changing dyanamics from a teriary core hospital in North India (2022)
20) Uma Maheshwari S., M.N. Sumana (Retrospective analysis on distribution & antifungal Susceplibility profile of Candida in clinical samples : a study from southern india) (2023)
21) Maria Belen carrarafausto Sebastian cabczas, EdyrdoTejera, Antonio Machado – Precalence of bioflims in Candida spp. bloodstream infections : A meta ansalysis. (2022)
22) David olusogaogbolu, Anthony Alaba Oni Oluwole Adebayo Daini, AP oloko Journal of medicinal food 10(2), 384-387, 200)
23) L. Julia Douglas Candida biofilms & their role in infection. (2003)
24) Clarissa J. Nobile, Jeniel E. Nett, David R. Andes, Aqron P. Mitchli Function of *Candida albicans* Adhesin Hwp1 in Biofilm formation. (2006

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